Supplementary Methods

Surface sterilization of eggs

Axenic larvae were produced by placing eggs into sterile Petri dishes containing 70% EtOH for 5 min, transferring to a solution of 3% bleach and 0.1% ROCCAL-D (Pfizer) for 3 min, transferring again to 70% EtOH for 5 min, and rinsing 3x in sterile water. Sterile water was produced by autoclaving.

Verification of sterility of axenic larvae and diet

Sterility of larvae was confirmed by culture-based and PCR analysis. A pool of 30 first instars from surface sterilized eggs were rinsed in sterile water and transferred to 100 ul of sterile PBS and homogenized. Larval homogenates were plated on Luria broth (LB) and brain-heart infusion (BHI) agar plates at 28° C for 24-72 h and subsequently checked for colonies. DNA was also isolated from three pools of 10 larvae using the Gentra Puregene Yeast/Bacteria Kit (Qiagen). Following DNA extraction, the 16S rDNA gene was amplified using either universal bacterial primers 27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') or *Wolbachia*–specifc primers WspF (5'TGGTCCAATAAGTGATGAAGAAACTAGCTA-3') and WspR (5'AAAAATTAAACGCTACTCCAGCTTCTGCAC—3'). For each mosquito species, three replicate 10 uL reactions were conducted, each containing 1 X HotMaster Taq Buffer, 200 uM of each dNTP, 0.2 uM of each primer, 1 U of Hotmaster *Taq* polymerase (5 Prime) and 0.8 uL of DNA template. Reactions were run on a MJ Mini thermocycler (Bio-Rad) with amplification cycle conditions as follows: denaturation at 94° C for 2 min, followed by 30 cycles of 94° C for 20 s, 50° C for 20 s, and 65° C for 1 min, and final extension at 65° C for 5 min. Products from the three replicate amplifications were pooled and 5 uL of the combined PCR product was electrophoresed on a 1% (wt/vol) agarose gel. The same methods were used to verify sterility of irradiated diet.